Gene-Foci Biotechnologies

Plasmid Miniprep Kit

- Catalog No. GF2701
- User's Manual
- For Research Use Only
- In vitro Use Only

Plasmid DNA Miniprep Kit

Catalog No.：GF2701

Catalog No.	Preps
GF2701-50	50
GF2701-200	200

❖ APPLICATIONS

Ideal for small scale (up to 20 μg) of molecular biology level plasmid DNA preparation.

❖ Kit Contents And Storage Conditions

Miniprep Kit	Storage Conditions	50 preps	200 preps
RNaseA（10mg/ml）	-20°C	150μl	500μl
Buffer P1	4°C	15 ml	50 ml
Buffer P2	Room Temp.	15 ml	50 ml
Buffer P3	Room Temp.	20 ml	70 ml
Wash Buffer WB	Room Temp.	15 ml Add ethanol before first use	50ml
Elution Buffer EB	Room Temp.	10ml	20ml
Spin Column AC	Room Temp.	50	200
2 ml Tubes	Room Temp.	50	200

This kit can be stored at room temperature for up to 12 months without showing any decrease in quality and yield.

❖ NOTES

1. Before use, add the provided RNase A solution to buffer P1 (final concentration 100ug/ml), gently mix well, and store at 2–8°C. If the RNase A in buffer P1 get inactivated due to long term or improper storage，there might be small amount of RNA contaminant in the extracted plasmid DNA. To avoid the RNA contamination, just add more RNase A to buffer P1.
2. When stored at low temperature, the SDS in buffer may precipitate out. To redissolve the SDS，just warm up buffer P2 in a 37°C water bath. To avoid bubbling, do not shake buffer P2 vigorously.
3. Recap the bottles right after use to avoid unexpected oxidation, evaporation and change of pH due to long term exposure to air.

❖ INTRODUCTION

The Gene-Foci miniprep kit provides a fast, easy and cost-efficient way of lab scale (up to 20 μg) plasmid DNA preparation. This kit use silica matrix technology to bypass the labor intensive steps of phenol-chloroform extraction and ethanol DNA precipitation. The Gene-Foci miniprep kit utilizes enhanced SDS-alkaline method to lyse *E.coli* host cells. The silica matrix in the spin column selectively binds plasmid DNA in the cell lysate under high salt, low pH condition, then the washing steps eliminate contaminants，finally, the pure plasmid DNA is eluted from the silica matrix under low salt, high pH condition.

❖ HIGHLIGHTS

1. High quality silica membranes guarantee the yield and consistency between different batches.
2. Unique protein cleaning up wash buffer ensures efficient clean up of residue DNase, even in the DNase enriched JM and HB010 cell lines.
3. Fast and toxic-free. DNA yield and purity is comparable with plasmid DNA extracted with branded counterparts. Extracted DNA can be directly used in most molecular biology applications, such as enzyme digestion, transformation, PCR amplification, in vitro transcription and Sanger sequencing.

❖ ATTENTION

1. All the steps are performed at room temperature, use microcentrifuge such as Eppendorf 5415D or similar model that can handle 13,000 rpm or higher speed.
2. Buffer P2 contains irritating chemicals, wear gloves when handling. Avoid direct contact with skin, eyes and clothes. If contaminated, rinse with large amount of water immediately.
3. The yield of plasmid DNA is correlated with cell density, copy number of the plasmid, etc. For regular high copy number plasmid, inoculate a freshly streaked single colony from a selective plate into 1.5-4.5 ml of liquid media containing appropriate antibiotics, culture overnight (14-16 hours) at 37°C with vigorous shaking. Up to 20 μg plasmid DNA can be obtained. For low copy number plasmid or plasmid more than 10 kb, use 5-10 ml overnight culture，also, increase the volume of buffer P1, P2 and P3 used in plasmid extraction correspondingly. Other steps remain the same.
4. The concentration of purified plasmid can be determined by agarose

electrophoresis. Alternatively, the concentration of plasmid DNA can be mesured by UV method, such as Nanodrop or spectrophotometer measurement.

5. To determine the size of a plasmid by electrophoresis, the plasmid should be linearized by restriction enzyme first. After agarose electrophoresis side by side with DNA marker, the size of the plasmid can be estimated by compared with the size of DNA marker. THE SIZE OF SUPERCOILED OR NICKED PLASMID DNA COULD NOT BE DETERMINED BY COMPARED WITH THE SIZE OF LINEARIZED DNA MARKER DUE TO THE DIFFERENT MIGRATION PROPERTY!

6. Elution buffer EB does not contain chelator EDTA, thus minimizes the effect on downstream experiment such as enzyme digestion, ligation. Alternatively, the plasmid DNA can be eluted with water. However, to ensure efficient elution, the pH of the water must be equal or higher than 7.5. Plasmid DNA should be stored at -20°C.

❖ PROTOCOL: Isolation of Plasmid DNA Using the Gene-Foci® Miniprep Kit

Hints：

⇨ **Before start, add the indicated amount of ethanol into buffer WB and buffer PE, mix well, and mark the bottle with a check.**

⇨ **Add the whole vial of RNase A into buffer P1, mix well. Store buffer P1 with RNase A at 2-8℃ after each use.**

⇨ **Precool buffer P3 on ice before start.**

1. **Inoculate a freshly streaked single colony from a selective plate into 1.5-4.5 ml liquid LB medium containing appropriate antibiotics, culture overnight (14-16 hours) at 37°C with vigorous shaking.**
2. **Harvest the cells by centrifugation at 12,000 rpm for 30 seconds in a desktop centrifuge.Decant or aspirate the supernatant without disturbing the cell pellet.**
3. **Resuspend the cell pellet with 250μl buffer P1, vortex vigorously to ensure complete resuspension.**
4. **Add 250 μl Buffer P2 and gently invert the tube 4–7 times to mix well，incubate at room temperature for 4 minutes.**

   Mix gently, do not vortex. As vigorous vortexing may result in gnomic DNA shearing. Do not allow the lysis reaction to proceed for more than 5 minutes.
   Buffer P2 contains irritating chemicals, wear gloves when handling. Avoid direct contact with skin, eyes and clothes. If contaminated, rinse with large amount of water immediately.
5. **Add 350μl ice-cold P3，immediately but gently invert the tube 4 -7times to mix well. Incubate on ice for 3-5 minutes to facilitate precipitation，then spin at 13,000rpm for 10 minutes.**

   To avoid localized SDS precipitation, mix the solution immediately after adding Buffer N3.

6. **Carefully transfer the supernatant from step 5 into the provided spin column AC, centrifuge at 12,000rpm for 30-60 seconds, discard the flow-through.**

7. **Wash the spin column AC by adding 0.7 ml buffer WB and centrifuging at 13,000rpm for 30 seconds. Discard the flow-through. Repeat the wash step one more time with 0.5 ml buffer WB.**
   Make sure ethanol has been added into buffer WB.

8. **Discard the flow-through, put the spin column AC back into the 2ml centrifuge tube, spin at 13,000rpm for additional 2 minutes to remove residual wash buffer.**
   Residual ethanol in buffer WB may inhibit subsequent enzymatic reactions such as restriction enzyme digestion and ligation.

9. **Transfer the spin column AC into a clean 1.5 ml centrifuge tube. To elute, add 50-100µl elution buffer (buffer EB) to the center of the silica membrane in the spin column, (for better yield, pre-warm up buffer EB to 65-70°C in a water bath), incubate at room temperature for 2 minutes，and centrifuge at 12,000 rpm for 1 minute. For better yield, re-apply the eluate into the column, repeat the spin.**

❖ TROUBLE SHOOTING

Problem	Possible causes and suggestions
Low yield	*Forgot to add antibiotics to the culture, plasmids are lost by the host cells-**Suggestion:** make sure appropriate antibiotics is added into solid and liquid media. *Cells have cultured for more than 16 hours, bacteria start to lyse-**Suggestion:** Inoculate freshly streaked single colony into liquid media with appropriate antibiotics, shaking culture for 12-16 hours. *Low copy number plasmid is used-**Suggestion:** Use high copy number plasmid whenever possible, for low copy number plasmid, use larger culture volume. *Insufficient culturing time, A_{600} is too low-**Suggestion:** do not harvest the cells until A_{600} reaches 2-4. *Insufficient cell lysis-**Suggestion:** Do not use more volume of cell culture than suggested; when resuspend the cells in buffer P1, vortex vigorously to make sure the cells are well resuspended, no visible cell clumps should be visible. *SDS in buffer P2 precipitated out of solution-**Suggestion:** Before Adding Buffer P2, check the P2 bottle to make sure there is no SDS precipitation. If SDS precipitated out, warm up buffer P2 in 37°C water bath to redissolve. *Poor elution-**Suggestion:**Please refer to Attention #6 and protocol step 10 for suggestions。
No DNA	*Forgot to add ethanol into buffer WB -**Suggestion:** Make sure the appropriate amount of ethanol is added into buffer WB.

Problem	Possible causes and suggestions
Plasmid DNA is resistant to enzyme digestion	*Skipped step 9, ethanol in DNA eluate-**Suggestion:** Spin dry the column as indicated in step 9, then air dry the column for several minutes before elution. *Silica fines in eluate-**Suggestion:** Spin the eluate at 13,000rpmfor 1 more minute, use the supernatant for enzyme digestion.
Plasmid DNA degraded	*Nuclease contamination-**Suggestion:** Make sure wash step 6 is performed to get rid of trace nuclease activity.
Genomic DNA found in eluate	*Genomic DNA is sheared during cell lysis step-**Suggestion:** After adding buffer P2, mix gently to avoid genomic DNA sheaing.
Nicked or denatured plasmid DNA band in electrophoresis	*Cell lysis step too long-**Suggestion:** Do not lyse cells more than 5 minutes.
RNA in the eluate	*Forget to add RNase A to buffer P1-**Suggestion:** Make sure RNase A is added to buffer P1 before use. *Too large Culture volume-**Suggestion:** Do not use culture volume more than suggested. *RNase A inactivated-**Suggestion:** If the buffre P1 containing RNase A has been stored for more than 3 months, add more RNAaseA.

Ordering Information

To order Gene-Foci products, please try the following methods:

(1) Order online

Register for an account on www.Gene-Foci.com, login, and place your order using our shopping cart and secure online checking out system.

(2) Call our toll-free number +1-888-315-9018

(3) Send Email to order@Gene-Foci.com

(4) Fax your order to +1-888-959-0868

To expedite your order, please provide the following information:

Customer user name

Purchaser's name and detailed contact information

Purchase Order Number (If any)

Billing address

Billing information

Shipping address

Description of the order